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A NEW SERUM FACTOR IN PATIENTS WITH RHEUMATOID ARTHRITIS THE ANTIPERINUCLEAR FACTOR*

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During an investigation of the occurrence of antinuclear factors (ANF) in the serum of patients with systemic lupus erythematosus (SLE), rheumatoid arthritis, ankylosing spondylitis (Bechterew's disease), and various other diseases, by means of Coons's immunofluorescent technique with epithelial cells from human buccal mucosa as substrate, we found (in addition to the variations in nuclear fluorescence as described by Beck, 1961) a cytoplasmic fluorescence due to a factor which, as far as we know, has not previously been described.

In epithelial cells from healthy human buccal mucosa first incubated with a serum containing the factor and thereafter layered with a rabbit antihuman globulin serum conjugated with fluorescein isothiocyanate, a speckled cytoplasmic fluorescence was observed.

This phenomenon was seen in some cells as brilliant fluorescent particles with a diameter between 4 and 7 mμ, often lying in a plane like the rings of Saturn around the nuclei. For this reason we called it perinuclear fluorescence (PNF) and the factor causing it the antiperinuclear factor (APF) (Figure, opposite).

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Nearly all the sera containing this factor turned out to be derived from patients with rheumatoid arthritis.

Preliminary studies suggested that the factor was an antibody against keratohyaline granules present in normal human buccal mucosa cells.

Materials and Methods

We followed the technique described by Mandema, Pollak, Kark and Rezaian (1961). Smears of fresh unfixed epithelial cells from healthy human buccal mucosa were used as substrate. The labelled antiglobulin serum used was a commercial globulin fraction of rabbit antihuman globulin serum conjugated with fluorescein isothiocyanate (Sylvana Chemical Co.).

The slides were inspected under a Reichert-Zetopan microscope using an Osram HBO 200 mercury vapour lamp as light source.

Results

(1) The Occurrence of the Antiperinuclear Factor (Table I)

Among 105 patients with definite rheumatoid arthritis (ARA criteria), 51 carried the factor and 54 did not.

Among 51 patients with possible and probable rheumatoid arthritis, six were positive.

TABLE I
ANTIPERINUCLEAR FACTOR IN 241 PATIENTS AND 431 CONTROLS

Diagnosis		No. of Patients	APF Positive		APF Negative	
			No.	Per cent.	No.	Per cent.
Rheumatoid Arthritis	Definite	105	51	48·5	54	51·5
	Probable and Possible	51	6	11·7	45	88·3
Ankylosing Spondylitis		42	—	—	42	100
Systemic Lupus Erythematosus		12	1	8·3	11	91·7
Rheumatic Fever		10	—	—	10	100
Degenerative Joint Disease		21	—	—	21	100
Control Population of Schiermonnikoog		431	4	<1	427	>99

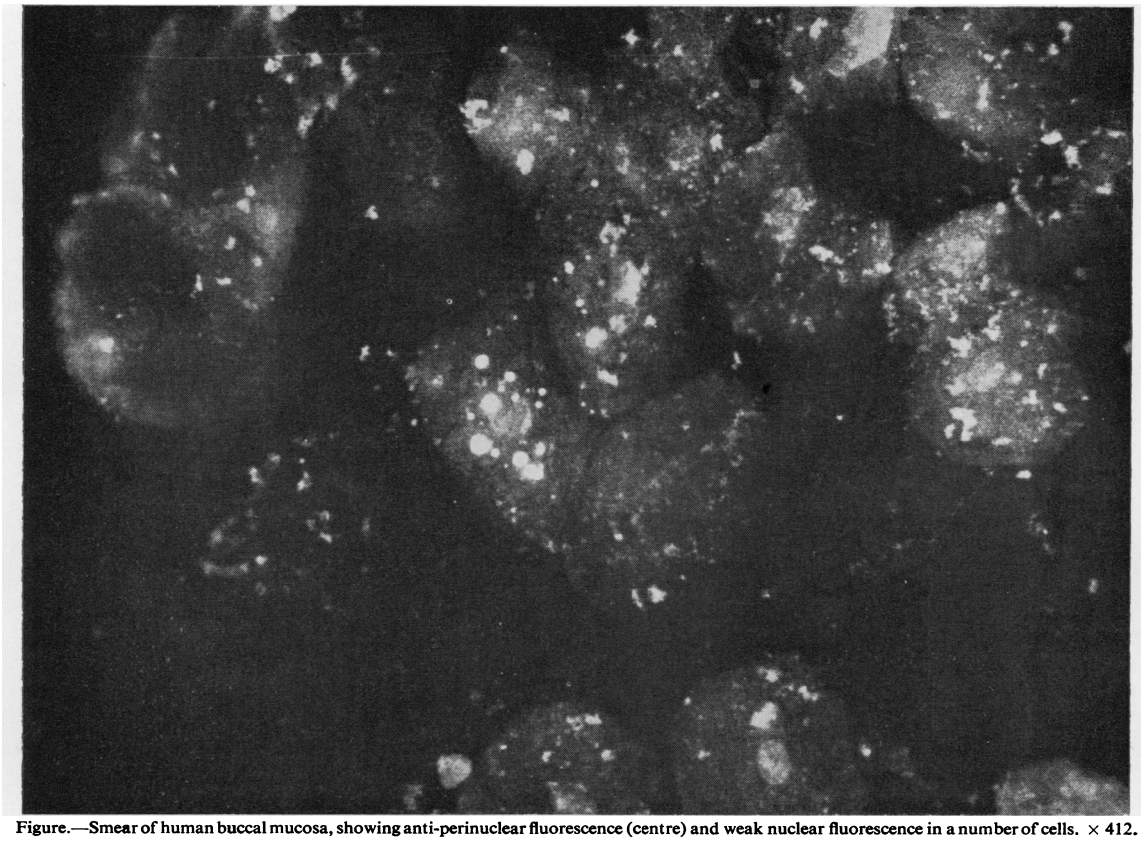

Figure.—Smear of human buccal mucosa, showing anti-perinuclear fluorescence (centre) and weak nuclear fluorescence in a number of cells. × 412.

None of 42 patients with ankylosing spondylitis were positive, although peripheral joint symptoms were also present in nineteen of them.

Negative results were also obtained in ten patients with rheumatic fever and 21 patients with degenerative joint disease.

Only one out of twelve patients with SLE was APF-positive, and this particular patient had serious joint involvement besides a number of other symptoms.

Controls.—Among a control series of 431 sera obtained in a study of the population of the coastal island of Schiermonnikoog, only four (<1 per cent.) carried the factor. The incidence of rheumatoid arthritis on this island was studied by de Blécourt and Westendorp Boerma (1963), who found eight patients with definite rheumatoid arthritis; three of these were included in the four whose sera were APF-positive.

Although more data are required, the present results suggest that the antiperinuclear factor has a high specificity for rheumatoid arthritis.

Of the 51 patients with definite RA who carried the APF, and 14 additional cases also with definite RA and APF, a total of 65 (see Table II), 56 had a positive Waaler-Rose test and/or a positive latex-fixation test, nine (14 per cent.) being negative in both tests (Table II).

TABLE II
WAALER-ROSE AND LATEX-FIXATION TESTS IN 74 APF-POSITIVE PATIENTS

Diagnosis		Waaler-Rose and Latex Test		Waaler-Rose Test		APF-Positive
		Positive	Negative	Positive	Negative	
Rheumatoid Arthritis	Definite	48	9	4	4	65
	Probable and Possible	6	2	—	—	8
Systemic Lupus Erythematosus		1	—	—	—	1

The factor was also present in fluid aspirated from the knees of rheumatoid arthritics with APF-positive serum, but not in fluid from the knees of patients with traumatic effusions.

Our studies have so far shown no positive correlation between APF-positivity and the age or sex of the patient, the ages of those in whom the factor was found ranging from 14 to 78 years. Nor has any positive correlation been found between the presence of the factor and the results of such laboratory studies as the E.S.R., haemoglobin concentration, the leucocyte and platelet count, the serum electrophoretic pattern, proteinuria, or the L.E.-cell phenomenon. We are trying to determine if there is any difference in clinical symptoms between APF-positive and APF-negative patients.

It is planned to carry out a study of the occurrence of the factor in the relatives of patients with rheumatoid arthritis.

(2) The Nature of the Perinuclear Factor

(*a*) *Substrate.*—The factor has hitherto been detected only when human buccal mucosa cells were used as substrate. With APF-positive sera we obtained negative results with tissue imprints from rat kidney and liver, with rat thyroid and stomach sections, and with guinea-pig liver, kidney, and adrenal sections. Sections from human cartilage and synovia were also negative, as were human vaginal smears obtained from fertile women (obtained from the Department of Gynaecology).

Human buccal mucosa cells from thirty healthy persons working in the Department of Medicine all showed brilliant perinuclear fluorescence with different APF-positive sera. These thirty donors included smokers and non-smokers and used six different kinds of toothpaste.

A serum containing the antiperinuclear factor showed perinuclear fluorescence in buccal smears from patients with rheumatoid arthritis and patients with SLE. Buccal mucosa cells from patients with rheumatoid arthritis carrying the antiperinuclear factor did not show perinuclear fluorescence when these cells were incubated with APF-negative sera, but they were positive with the patients' own APF-positive serum or other APF-positive sera.

These observations indicate that some epithelial cells from human buccal mucosa contain a compound which is either not present, or is present in a different form, in the other substrates so far studied.

It is not likely that the phenomenon is due to a contamination of the cells with some substance such as a virus from outside the cell. The results obtained with a particular test serum have remained the same over a period of 2 years both with buccal mucosa cells from the same person and with cells from other donors.

Electron micrographs of epithelial cells from human buccal mucosa show that these cells have in their cytoplasm 4 to 7 mμ granule-like particles with a distribution very similar to that of the perinuclear fluorescence. Using phase-contrast microscopy and comparing stained with unstained smears, it seems probable that these are the so-called "keratohyaline granules".

Electron microscopy did not show these granules in human vaginal epithelial cells.

Besides the perinuclear fluorescence, cells incubated with serum containing antiperinuclear factor also show a weak nuclear fluorescence, the significance of which is unknown. When such sera were absorbed with washed chicken red-cell nuclei before they were applied to the buccal mucosa cells, neither the brilliant perinuclear fluorescence nor the weak nuclear fluorescence was affected. On the other hand, antinuclear factor from sera derived from patients with SLE could be readily absorbed in the same procedure. The weak nuclear fluorescence accompanying the perinuclear fluorescence seems therefore, to be of a different nature from that seen with sera from patients with SLE.

(*b*) *Factor.*—Heating sera at 56° C. for 30 min. before applying them to the smears, had no effect on the prescence or brightness of perinuclear fluorescence.

The gammaglobulin nature of the factor was demonstrated by blocking experiments with conjugated antihuman albumin serum which showed no blocking, while unconjugated anti-gammaglobulin serum caused complete blocking of the perinuclear fluorescence.

The factor was not present in the macroglobulin fraction of the serum, as could be demonstrated by treating APF sera with doubly-distilled water at 4° C. overnight: after centrifugation the factor was present in the supernatant and not in the precipitate. Treatment of APF-positive sera with mercaptoethanol also failed to change the results.

Antiperinuclear factor is probably a 7S gammaglobulin, since unconjugated rabbit antihuman 7S gammaglobulin serum produced blocking and a rabbit antihuman B_{2A} and antihuman B_{2M} sera, both unconjugated, did not interfere with the perinuclear fluorescence.

Work on the isolation of the protein fraction containing the antiperinuclear factor has yet to be done.

Discussion

It seems likely that the antiperinuclear factor is an antibody of the 7S gammaglobulin type, directed

against the "keratohyaline granules" present in epithelial cells of human buccal mucosa. The nature of these granules is not known: they are usually thought to be precursor material in keratin formation. According to Albright (1960), however, their role in keratinization is doubtful. He states that they may have no connexion with this process because they apparently contain no sulphhydryl groups, disulphide bonds, or other substances known to constitute sequential ingredients in keratin formation.

The antiperinuclear factor does not seem to be related to the anticytoplasmic factors found in sera from patients with SLE and from cases of primary biliary cirrhosis and Sjögren's syndrome exhibiting complement-fixation reactions with cytoplasmic cellular constituents.

In these complex studies by Deicher, Holman, and Kunkel (1960), 29 sera from rheumatoid arthritics showed no reaction at higher titres and the incidence at low titres reactions was but slightly above normal. Approximately the same percentage of low titre reactions were found among the miscellaneous hypergammaglobulinaemic sera. Thus the distribution of the occurrence of APF is entirely different from that of the anticytoplasmic antibodies described by these authors.

A number of additional experiments are required to determine the nature of the antiperinuclear factor and the substance on which it reacts.

Summary

Using Coons's indirect immunofluorescent technique with epithelial cells from healthy human buccal mucosa as substrate, a new factor was found in the sera of about 50 per cent. of patients with rheumatoid arthritis.

This factor is called antiperinuclear factor because it gives a perinuclear fluorescence. It is probably an antibody of the 7S gammaglobulin type against keratohyaline granules in buccal mucosa cells.

The incidence of the factor in healthy people seems to be very low, and its presence in other diseases so far studied, also seems to be very rare.

It should be noted that the factor was not present in the serum of 42 patients with ankylosing spondylitis.

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Un nouveau facteur sérique chez des malades atteints d'arthrite rhumatismale, le facteur antipérinucléaire (APF)

RÉSUMÉ

A l'aide du procédé d'immunofluorescence indirecte de Coons et des cellules épithéliales de la muqueuse buccale humaine saine, utilisée comme substrat, on a trouvé un nouveau facteur dans les sérums d'environ 50 pour cent des malades atteints d'arthrite rhumatismale.

On appelle ce facteur antipérinucléaire parce qu'il emet une fluorescence périnucléaire. Il s'agit probablement d'un anticorps du type gammaglobuline 7S contre les granules kératohyalines dans les cellules de la muqueuse buccale.

La fréquence de ce facteur chez des personnes saines semble être très basse, et sa présence en d'autres maladies étudiées jusqu'à présent semble aussi être très rare.

On remarque que ce facteur n'était pas présent dans les sérums de 42 malades atteints de spondylarthrite ankylosante.

Un nuevo factor sérico en enfermos con artritis reumatoide, el factor antiperinuclear (APF)

SUMARIO

Empleando el procedimiento de inmunofluorescencia indirecta de Coons y las células epiteliales de la mucosa bucal humana sana como substrato, se encontró un factor nuevo en los sueros de alrededor de un 50 por ciento de enfermos con artritis reumatoide.

Este factor se llama antiperinuclear porque produce fluorescencia perinuclear. Se trata probablemente de un anticuerpo del tipo gamaglobulina 7S contra los gránulos queratohialinos en las células de la mucosa bucal.

En personas sanas este factor se encuentra con poquísima frecuencia y en otras enfermedades estudiadas hasta la fecha su presencia también parece muy rara.

Se nota que este factor fué ausente en los sueros de 42 enfermos con espondilartritis anquilosante.